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Water Damage
June 1993

The Pennsylvania State College
The Graduate School
Department of Agricultural and Biological Chemistry

A Study of the Arsenic Content of
Pennsylvania Tobacco and Tobacco Soils

A Thesis
by
Lewis Wallace Ilgen

Submitted in Partial Fulfillment
for the degree of
Master of Science
August 1937

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I. Introduction

During recent years a number of investigations have been made in order to find out if more than the ten essential elements are needed for normal plant growth. The effect of compounds of arsenic upon plant growth has been a subject of scientific investigation.

It has been recognized that arsenic is quite generally found in animal tissue; and since the animal must have obtained the arsenic from its food, it was soon found that arsenic is present in minute amounts in many plants. Many investigations show that arsenic is widely distributed in the soil, both derived from the original rock mineral as pyrite and artificially added as insecticides.

Since arsenic is found to be commonly present in animal and vegetable tissues the question was raised as to whether or not arsenic is essential to the life processes. It is not yet definitely known that minute quantities of substances such as arsenic may have a very decided effect on these processes. The more recent investigators show that it is still too early to make any definite and final conclusion as to the rôle in which arsenic plays in plant and animal life.

If minute quantities of arsenic may or may not be useful to plant and animal life, it has been often evident that it is a deadly poison to both animal and plant life if the quantity consumed is large.

More recent research on methods for the determination of minute quantities of arsenic present in biological material have been developed and are being further developed. By the use of these refined, delicate and accurate methods more research will be done on finding the rôle of arsenic in biological systems.

II. Review of Literature

Arsenic has been found to be a common element of all agricultural soils; varying in quantity from a mere trace to concentrations sufficient to inhibit plant growth.

Greaves (13, 14) found arsenic present in all soils that were analyzed. Even in virgin soils arsenic was found to the extent of 4 ppm. It did not result from smelter smoke, or similar sources as spray and dust residues, and, as reported by Headden (15) in his investigations, was derived in this case from the weathering of original rocks from which the soil was formed.

Other investigators (15, 2, 18) found arsenic present in varied quantities in the soil due to the fact that arsenic not only occurs in the soil but because of methods of application and fixation by bases, tends to accumulate in the surface soil.

Headden (15) found arsenic in some cultivated orchard soils to the extent of 138 ppm. and he believes that in many places the accumulation is so great as to render it injurious to vegetation.

Some investigators refer to arsenic as a "normal constituent of the soil" because it is almost always present in soils. Kunkel (17) found arsenic in many rocks and waters. Foreign investigators have shown it to be present in appreciable quantities in the surface and underground water of some regions.

Plants grown on soils containing arsenic were found to contain arsenic. Beadden (15) found arsenic present in Alfalfa when grown on soil containing arsenic. Other investigators have traced arsenic from the soil to the plant (29), 4, 16, 21, 28). The largest source of soluble arsenic for plant growth is from sprays and dusts, (20).

It has been shown that arsenic renders both beneficial and harmful effects to plant growth. Cooper (10) has found that the application of small quantities of arsenic to light soils decreased the yield of cotton and cowpeas. On the other hand Stewart and Smith (29) produced no injury to vegetables until large quantities of soluble arsenic were added to a highly calcareous soil. Arsenic used as insecticides finds its way to the soil and causes damage to vegetation, (1, 16, 24).

Arsenic as a nutrient element is thought to be essential for plant growth. Spencer (28) found arsenic to be toxic at medium concentrations in sand cultures but gave 100% germination.

Remington (25) and Sheldon (26) have made studies of the presence of arsenic in the animal body and its toxic effects. These investigators have traced the arsenic accumulation in animal tissue from plants. Billetr and Marfurt (7) made a study of the content and localization of arsenic in the body. They found an average of 0.0103 mg. per 100 g. of body tissue.

The greatest source of arsenic in the body is from vegetation which has been sprayed or dusted with arsenical insecticides and fungicides.

Cassil and Smith (9) made a number of analysis of various tobacco products and found them to contain varied quantities of As_2O_3 , from 4.1 to 36 PPM.

Spallino (27) and Popp (23) found that tobacco from certain countries contained more arsenic than samples from others. Since the use of arsenical insecticides is not widespread on tobacco, it would appear that the arsenic was derived from the soils of the areas on which these tobaccos were grown.

Since the arsenic found in animal and plant tissue is derived largely from soil sources, a more detailed study of soil arsenic was made by several workers. Albert and Arndt (3) found that the amount of arsenic present in a field soil is not necessarily related to the toxicity toward crops. Some fields on which normal crops can be grown may contain as much arsenic as a near by field on which similar crops may be a complete failure. Greaves (13) states that the toxicity of arsenic to plants is probably governed by its solubility in the soil.

Some investigators have correlated the water soluble with the arsenic present in the plant, while others have correlated acid soluble and in some cases the total of the soil with that of the plant.

III. Object and plan of Investigation

It is conceived by plant physiologist that a plant takes up what elements are present in the soil, whether beneficial or toxic to the plant. Some elements tend to be accumulated in the plant. This may be due to the nature of the plant or due to the activity of the element itself. Plants that are used for food or for any other purpose that will lead to an accumulation of arsenic in the body should be used with caution.

The tobacco plant being a plant that is a heavy mineral feeder and a carrier of arsenic, and is utilized for smoking and chewing, should be grown and handled as to keep the arsenic content to a minimum.

The object of this investigation is to find the amount and availability of arsenic in the soil, and the amount and utilization of arsenic by the tobacco plant.

The problem resolves itself in the correlation of total arsenic in the plant as related to either the total arsenic or arsenic soluble in 0.05N HCl (acid soluble) or water soluble arsenic in the soil. In short it is an attempt to determine if the total arsenic or the soluble arsenic in the soil influences the amount taken up by the tobacco plant.

The analytical work consists of the analysis of twenty four tobacco soils, two each on twelve farms for total, acid soluble and water soluble arsenic, and the analysis of

representative samples of tobacco grown on the twenty four soils.

These twenty four soils are located on twelve farms, well distributed over Lancaster County, Pennsylvania. Each of these twelve farms has two samples, on A and B sample taken from different areas on the farm. The A fields were treated with potassium carbonate while the B fields were treated with potassium sulfate.

This is also an attempt to show a variation in the arsenic content of Lancaster soils and to show, if any, a variation of arsenic in different fields on the same farm.

No arsenic was added to these soils or to the tobacco plants during the year of 1936.

Sample of soil and tobacco were attained at the same time so the conditions at the time of sampling are comparable. Sampling was done on August 25, 1936.

IV. Methods

Preparation of samples for analysis

When determining arsenic in biological material as soil and plant materials, all organic matter must be removed by oxidation and the arsenic must be kept in the oxidized state in order to keep the losses of the element to a minimum.

The organic matter is oxidized from plants and soils by digestion with conc. HNO_3 and conc. H_2SO_4 with constant heating. All oxides of nitrogen must be removed and the material should be water - clear. This is done in a 500 cc Kjeldahl flask. After this digestion the sample is then transferred to a volumetric flask from which suitable aliquots can be taken.

Acid soluble arsenic was obtained by leaching the soil with a solution of 0.05 N, HCl. Ratio of soil to acid solution used is 1:10.

Of all the methods available for the determination of arsenic only a few are suited for the determination of minute quantities of arsenic present in soil and plant materials.

The Gutzeit and the Bromate methods are best suited, the former being the best for very small quantities of arsenic. Callon and Clifford (8) made a comparative study of these two methods. They found that these methods were quite comparable when slight changes were made in the procedure of the Bromate method. The Bromate method is

more reproducible than the Gutzeit when the quantity of arsenic present in the sample is over 0.020 mgm., while under this amount the Gutzeit method is better.

In this work the Gutzeit method is used. The accuracy of this method is given by Barner and Murray (6) who states the probable error of the mean of duplicate strips is 0.0039 mgm. The probable error is small when the quantity of arsenic present is small. Neller (22) made a study of the accuracy of this method by varying physical factor and reagents to get the most accurate results when using known solution of arsenic. According to Neller (22) the probable error for a series of determinations is given as 0.0015 Mg. of As_2O_3 . Frear and Hodgkiss (12) studied the accuracy of this method in connection with the determination of arsenical residues on apples. The average derivation obtained was 0.00070 grains for duplicate samples.

The Gutzeit Method

The Gutzeit method is given in the A.O.A.C.(5) as follows: An aliquot of the prepared samples (organic matter removed and the arsenic in an oxidized state) the arsenic is reduced to an arsenic (AsH_3) in the Gutzeit generator by the action of metallic zinc, SnCl_2 , KCL and HCL. All reagents must be arsenic free. The evolved gas is passed through a drying tube, then past a strip of paper sensitized with a standard solution of HgBr_2 . The amount of arsenic present is determined by the length of brown-to-tan stain formed on the sensitized paper. A set of standard strips is made with varying quantities of a standard arsenic solution. The length of the stain of the unknown can be compared with a known stain.

V. Presentation of Data

Table 1.

Amounts of arsenic (as As_2O_3) in the soil and in tobacco plants grown on the same soil.

A - Treatment

Farm No.	Arsenic in the Soil		Arsenic in the Plant
	Total	Acid Soluble (Soluble in 0.05N HCL)	Total
	PPM	PPM	PPM
1	17.5	0.55	1.3
2	8.5	0.70	1.0
3	6.0	0.30	0.5
4	9.5	0.45	0.9
5	13.5	0.50	1.2
6	14.3	0.35	1.0
7	8.3	0.37	1.1
8	18.4	0.25	0.9
9	6.0	0.45	1.6
10	22.6	0.55	2.2
11	11.0	0.30	1.2
12	18.5	0.25	0.9

Table 2.

Amounts of arsenic (as As_2O_3) in the soil
and in the tobacco plants grown on the same soil.

B - Treatment

Farm No.	Arsenic in the Soil		Arsenic in the Plant
	Total	Acid Soluble (Soluble in 0.05N HCL)	Total
	PPM	PPM	PPM
1	14.0	0.55	1.0
2	12.3	0.75	1.5
3	4.5	0.30	0.5
4	7.5	0.50	1.3
5	13.0	0.20	0.5
6	14.6	0.40	1.0
7	9.0	0.20	0.5
8	18.4	0.40	0.8
9	13.5	0.50	1.25
10	17.5	0.50	1.50
11	9.5	0.45	1.10
12	13.2	0.25	1.10

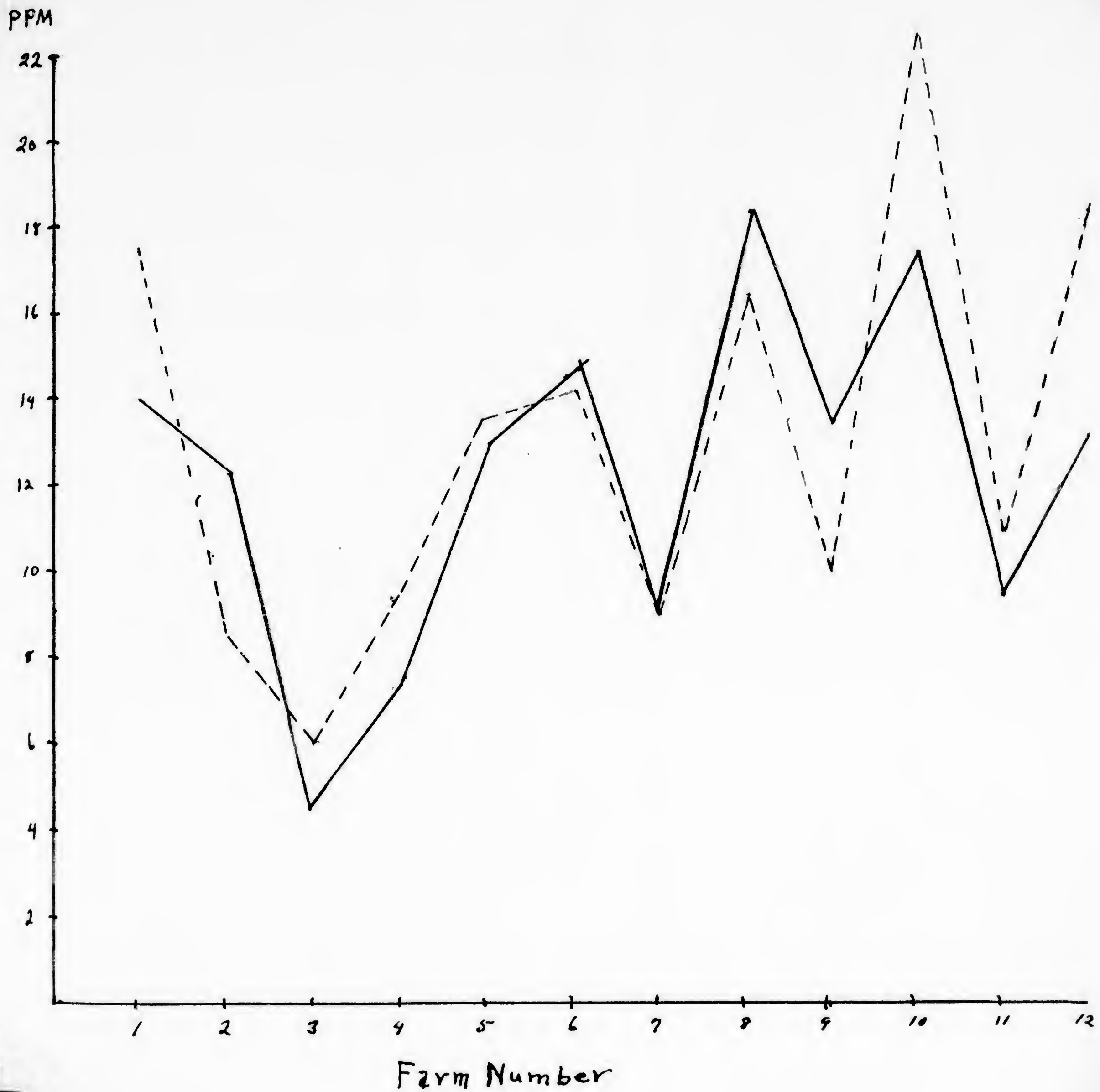


Figure I

Amounts of Total Arsenic (as As_2O_3) in the
Soil of Two Fields on the Same Farm.

A - Soils -----

B - Soils _____

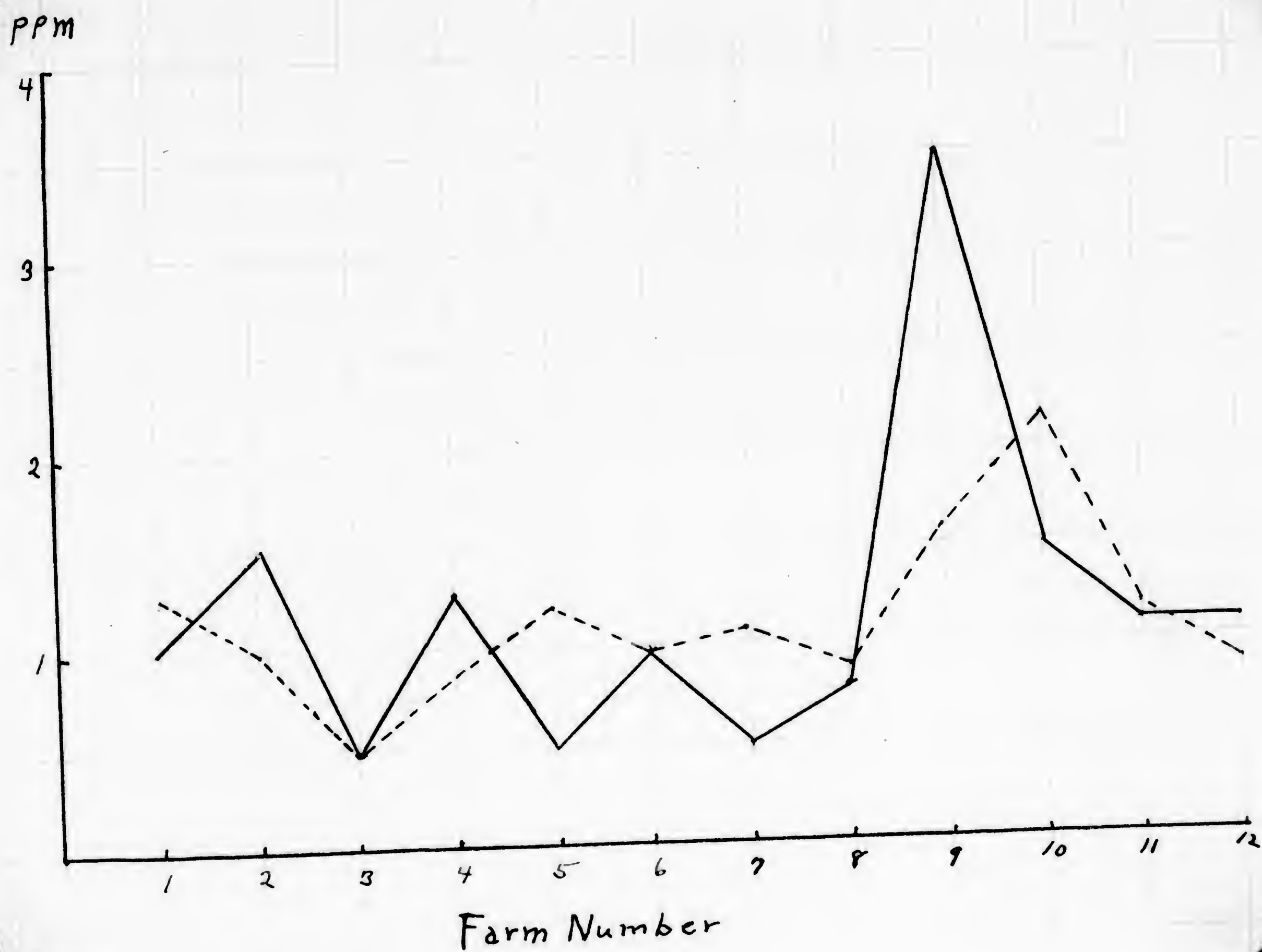


Figure II
Amounts of Arsenic (as As_2O_3) in the Tobacco
Plant grown on two different Fields on the same Farm.

A - Tobacco - - - - -

B - Tobacco - - - - -

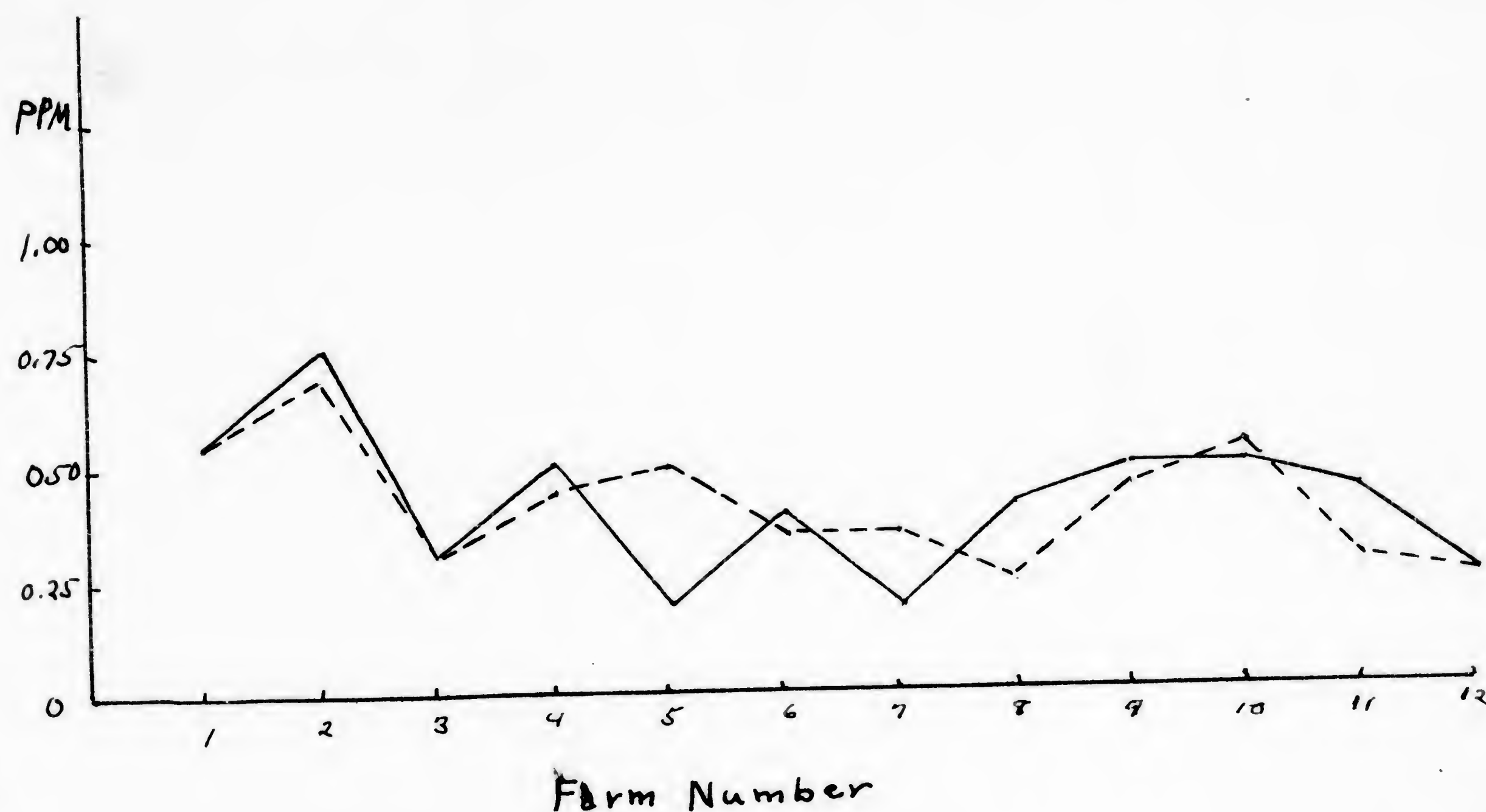


Figure III

Amounts of Acid Soluble (Soluble in 0.05N HCL)
Arsenic (as As_2O_3) in the Soil of Two Fields on the same
Farms.

A - Soils -----

B - Soils _____

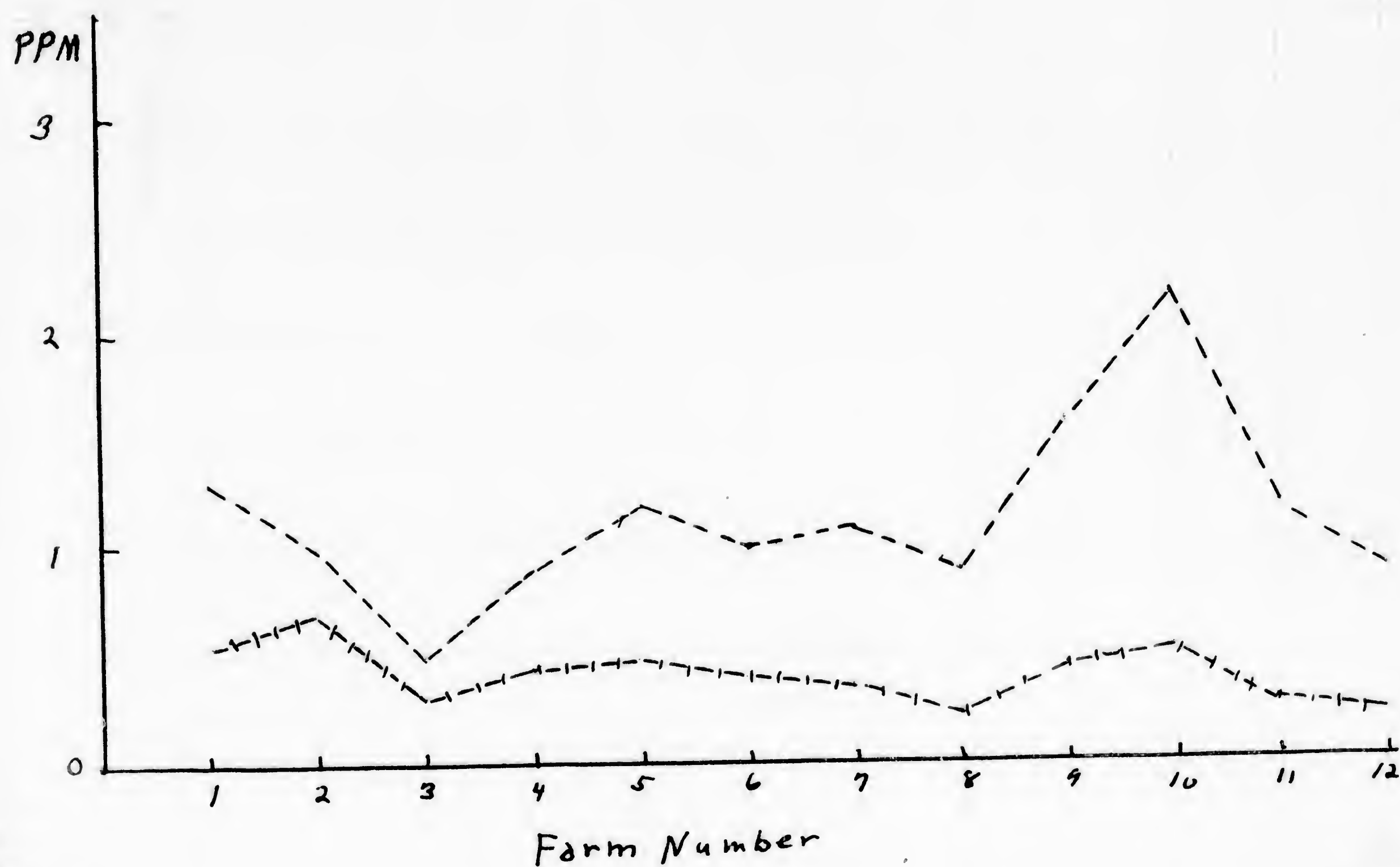


Figure IV

Amounts of Arsenic (as As_2O_3) in the Tobacco Plant
and the Acid Soluble (soluble in 0.05N HCL) Arsenic in the
A - Soils.

Plant - - - - -

Soil - - - - -

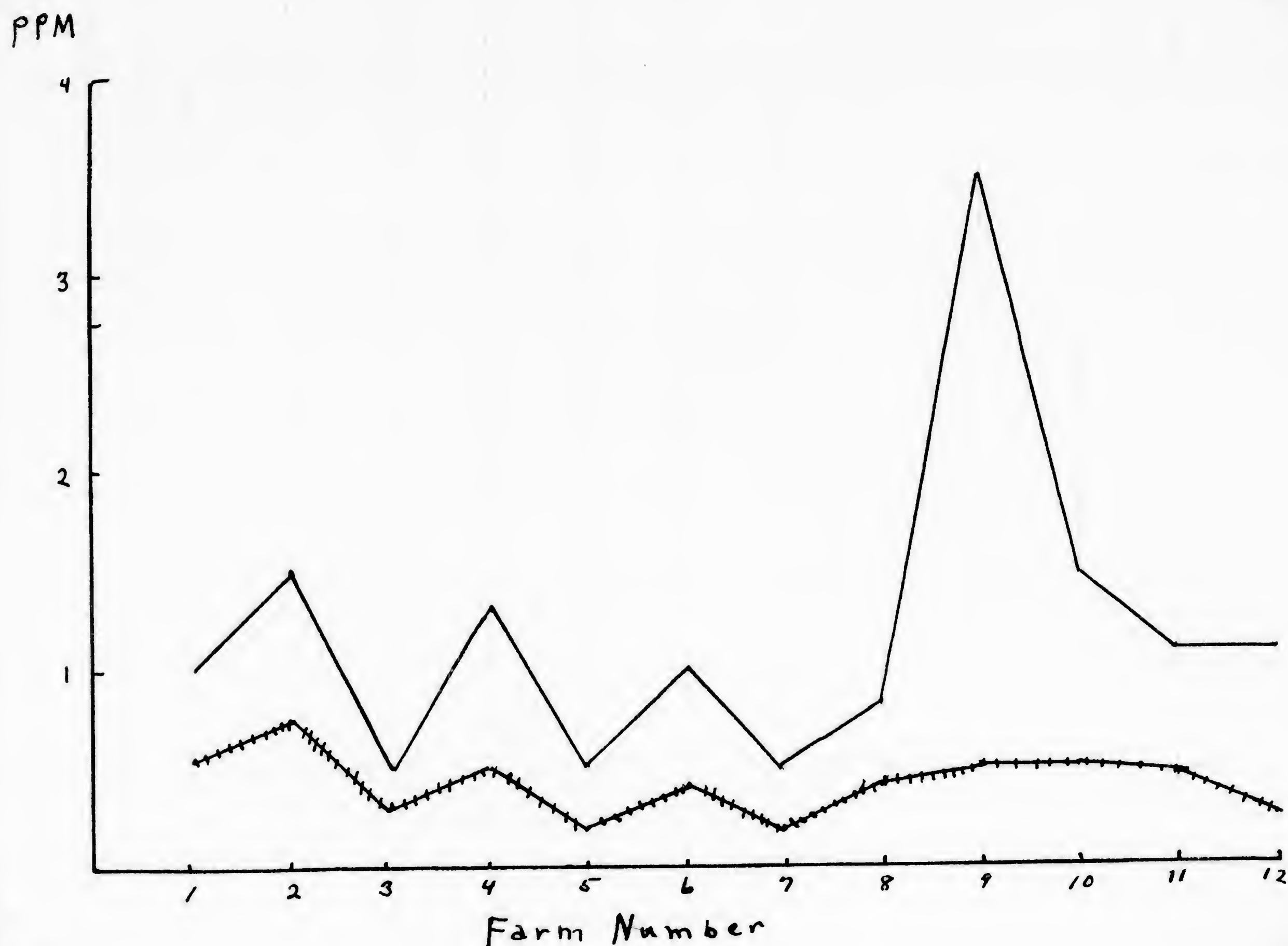


Figure V

Amounts of Arsenic (as As_2O_3) in the Tobacco Plant
and the Acid Soluble (soluble in 0.05N HCL) Arsenic in the
B - Soils

Plant —————

Soil ++++++

VI. Discussion of Results

The total arsenic of the soil varied from 4.5 to 22.6 parts per million or 9.0 to 43.2 pounds per acre of soil (2,000,000 pounds). Arsenic soluble in 0.05N HCL was low in comparison with the total, ranging from 0.20 PPM to 0.75 PPM, while the water soluble was present only in ~~traces~~. Water soluble arsenic was too small in quantity to be determined.

The total arsenic of the tobacco plant varied from 0.5 PPM to 3.5 PPM. The arsenic content of the plant was found to be a direct function of the acid soluble arsenic of the soil.

The correlation coefficient between acid soluble arsenic and total arsenic in the plant has been calculated (55) according to the formula.

$$r = \frac{\frac{\sum D_x \cdot D_y}{n} - (C_x \cdot C_y)}{G_y \cdot G_x}$$

Where r is the correlation between the two variables X and Y .

The probable error has been calculated by the formula:

$$P.E. = \frac{0.6745 (1 - r^2)}{n}$$

The correlation coefficient between the acid soluble arsenic and the total in the plant = 0.537 ± 0.0125 , while the coefficient between the total arsenic of the soil and the total of the plant = 0.331 ± 0.0089 . Both of the correlation coefficients are significant in the light of their probable errors, indicating that there is actually a relationship between the arsenic (both total and acid soluble) in the soil and that of the plant. But there is a close correlation between the acid soluble arsenic and that of the plant as shown by the larger correlation coefficient.

Table 3

Summary of Total Arsenic of Soil and Acid Soluble Arsenic of Soil

	Total Arsenic of Soil	Acid Soluble Arsenic of Soil
Maximum	22.6 PPM	0.75 PPM
Mean	12.3	0.413
Minimum	4.5	0.20
Ratio	30:1	

These figures are not from the same soil and plant of the same farm. They are the highest or lowest of all data considered. The mean is the average of twenty-four samples in all cases. The ratio is obtained by using the means.

Table 4

Summary of Plant and Total Arsenic of Soil

	Total Arsenic in Soil	Plant Arsenic
Maximum	22.6 PPM	3.5 PPM
Mean	12.3	1.117
Minimum	4.5	0.5

Coefficient of correlation 0.3310 ± 0.0089

Ratio 1:10.5

Table 5

Summary of Acid Soluble Arsenic and Plant Arsenic

	Acid Soluble Arsenic	Plant Arsenic
Maximum	0.75 PPM	3.5 PPM
Mean	0.413	1.117
Minimum	0.20	0.50

Coefficient of Correlation 0.532 ± 0.0125

Ratio 1:2.83

The ratio of the total arsenic content of the soil to that of the plant is roughly 1 : 10 while the total of the soil to acid soluble in the soil is 1 : 30 or only one-thirtieth of the total available for the tobacco plant. The ratio of the acid soluble to that of the plant is 1 to 2.83, which shows that the soluble arsenic is related in quantity to the amount in the plant.

The data obtained can be more clearly understood by referring to the graphs (1-5).

The data were not correlated with any other data taken from either the soils or the tobacco. The difference of the pH of the A and the B treated soils was not significant to mention, for this was the primary reason for both the A and B treatments on the same farm. The bacterial content of the soils showed no correlation.

VII Summary and Conclusion

1. The total arsenic content of the soil is not always a measure of the arsenic in the plant.
2. The arsenic utilized by the tobacco plant is closely related to the acid soluble arsenic of the soil.
3. Water soluble arsenic was too low in quantity to be measured quantitatively.
4. The arsenic content of the tobacco plant was more closely correlated with the acid soluble than the total arsenic of the soil. Therefore, the acid soluble, in this case, was the more available source of arsenic for plants.
5. The average amount of total arsenic found in Lancaster County soils is 12.3 PPM, acid soluble (soluble is 0.05N HCL) 0.413 PPM. The average arsenic of the tobacco from these soils is 1.17 PPM.
6. The ratio of total arsenic in soil to soluble in soil 1 : 30
Ratio of total in plant to soluble in soil 1 : 28.3
Ratio of total in soil to total in plant 1 : 10.5
7. The arsenic not only varied from farm to farm but also varied on the same farm. The A and the B soil treatments had a noticeable influence on the arsenic solubility of soil arsenic.
8. Neither the A or the B treated soils have any noticeable effect on the solubility of arsenic in the soil nor the utilization by the plant.

VIII.

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